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GRANT NUMBER DAMD17-96-1-6309

TITLE: Evaluation of a Self-Administered Intravaginal Swab for PCR Detection of Genitourinary Tract Infections Including Chlamydia, Gonorrhea, Trichomonas and Human Papillomavirus in Active Duty Military Women

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REPORT DATE: October 1997

TYPE OF REPORT: Annual

PREPARED FOR: Commander  
U.S. Army Medical Research and Materiel Command  
Fort Detrick, Frederick, Maryland 21702-5012

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19980130 179

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REPORT DOCUMENTATION PAGE			Form Approved OMB No. 0704-0188	
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1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE October 1997	3. REPORT TYPE AND DATES COVERED Annual (23 Sep 96 - 22 Sep 97)		
4. TITLE AND SUBTITLE Evaluation of a Self-Administered Intravaginal Swab for PCR Detection of Genitourinary Tract Infections Including Chlamydia, Gonorrhea, Trichomonas and Human Papillomavirus in Active Duty , , ,		5. FUNDING NUMBERS DAMD17-96-1-6309		
6. AUTHOR(S) Anne M. Rompalo, M.D.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)  The Johns Hopkins University Baltimore, MD 21205-2196		8. PERFORMING ORGANIZATION REPORT NUMBER		
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) Commander U.S. Army Medical Research and Materiel Command Fort Detrick, Frederick, Maryland 21702-5012		10. SPONSORING/MONITORING AGENCY REPORT NUMBER		
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION / AVAILABILITY STATEMENT  Approved for public release; distribution unlimited		12b. DISTRIBUTION CODE		
13. ABSTRACT (Maximum 200)  Sexually transmitted diseases (STDs) are among the major causes of morbidity for both men and women in the U.S. Comprehensive prevalence studies among military women for these infections have not been performed and STD medical facilities in the Army are limited. Implementation of an STD screening and treatment intervention strategy based on new, highly sensitive and specific diagnostic tests could have a multifaceted impact on Army women and on the medical system. This ongoing study is designed to assess the sensitivity and specificity of a self-administered intravaginal swab (SAS) for detection of gonorrhea, chlamydia, trichomonas, and human papilloma virus among active military women attending a health care facility at Fort Bragg, North Carolina. Of the 186 women who have volunteered for enrollment to date, 27% were Caucasian, 65% were African American, and their mean age was 25 years. The SAS compared to Standard Operation Procedure (SOP) diagnostics detected more cervical gonorrhea (2.7% vs 1.6%), more chlamydia (13.7% vs 12.6%), and all trichomonas infections detected either by SOP or culture. Furthermore, preliminary data using a "dry" SAS has resulted in equal, if not better, STD detection compared with the SAS shipped "wet" in transport media. This screening method is exciting since it may eliminate any biohazard transport or confidentiality problems and prove more cost-beneficial.				
14. SUBJECT TERMS Defense Women's Health Research Program		15. NUMBER OF PAGES 24		
		16. PRICE CODE		
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

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## Introduction

Sexually transmitted diseases (STDs) are among the major causes of morbidity for both men and women in the U.S. Among women serious sequelae such as pelvic inflammatory disease (PID), ectopic pregnancy, infertility, and cervical carcinoma can occur as a result of undiagnosed STDs (1). The direct medical care costs of these diseases are estimated to be in excess of five billion dollars annually (2). Infection rates for STDs in young, sexually active women between the ages of 18 and 30 may range from 5 to 25% (1). Since early infection may be asymptomatic in over half of the women, many do not seek health care until the development of complications.

Comprehensive prevalence studies among **military** women for these infections have not been performed, but small individual studies have demonstrated chlamydial infection rates of 10 to 15%, gonorrhea rates from 2 to 5%, trichomonas rates from 5 to 25%, and human papilloma virus (HPV) estimated rates ranging from 20 to 40% (3-7). These rates are substantially higher compared to 1993 Centers for Disease Control and Prevention (CDC) surveillance rates in the U.S. female population of 0.2% for chlamydia, and 0.14% for gonorrhea (8).

The diagnosis and treatment of STDs and their complications among active duty soldiers can result in substantial direct medical costs as well as disability and reduced deployability. STD prevalence and incidence can be effectively reduced by providing easy access to diagnosis and treatment. However, STD medical facilities in the Army are limited. Traditional approaches to STD diagnosis among women, even when trained medical and paramedical personnel are available, require a full gynecologic examination and laboratory diagnostic tests which are often not possible at the field level. Social impediments such as ostracization, embarrassment, and insensitive provider attitudes may prevent rapid access to care and lead to further clinical complications.

The recent development of molecular STD probes has revolutionized diagnostic approaches. These new tests, based on DNA technology and extensively evaluated in our laboratories, can be self-administered, can decrease the need for physical exam, are stable for weeks after obtaining specimens at room temperature, and are highly sensitive and specific. Implementation of an STD screening and treatment intervention strategy based on these diagnostic tests could have a multifaceted impact on Army women and on the medical system.

This proposal is designed to assess the sensitivity and specificity of a self-administered intravaginal swab (SAS) for detection of these STDs among active military women attending a health care facility at Fort Bragg, North Carolina.

## **Background**

New diagnostic assays for chlamydia and gonorrhea using molecular techniques have been developed over the past several years. In assessing sensitivity, it is generally accepted that culture provides the best sensitivity and specificity for most organisms. However, sensitivity and specificity may be variable depending on the pathogen cultured. In most settings, the diagnosis of gonorrhea is made most cheaply and reliably by culture. In addition, Gram stain diagnosis as presumptive evidence for gonococcal disease has a sensitivity greater than 95% in men, but is lower in women at approximately 60% (9,10).

For years, the diagnostic gold standard for chlamydia infection among both men and women was considered to be in-vitro cell culture. Chlamydia cultures, however, are expensive and may be only 85% sensitive in the best laboratories because of a variety of inherent limitations, including transport requirements, sample reliability, and infectious load (11). Gram stains of cervical and urethral discharge to detect increased numbers of polymorphonuclear cells is helpful to confirm a "syndromic diagnosis" that may be due to chlamydia, but sensitivity and specificity of Gram stain for chlamydia cervicitis are not optimal (12).

Consequently, a wide variety of more rapid and less expensive STD assays have been developed, especially for detection of chlamydia. These include enzyme immunoassays (EIAs) to detect chlamydial and gonococcal antigen in cervical secretions and urine; fluorescent monoclonal antibodies for the direct visualization of chlamydial elementary bodies on smears; rapid antigen tests designed for use in physician and STD clinics; and molecular probes including nucleic acid hybridization tests, polymerase chain reaction (PCR) and ligase chain reaction (LCR) techniques. With the exception of PCR and LCR, all nonculture methods of antigen detection for both gonorrhea and chlamydia are much less sensitive, detecting between  $10^3$  and  $10^4$  infectious particles per milliliter per sample. Sensitivities range from 60% to 85%, and some tests may also give false-positive results. PCR and LCR, however, have been shown to detect 1 to 10 infectious particles per sample and have sensitivities above 90% with almost 100% specificity.

The commercial PCR assay (Amplicor, Chlamydia; Roche Diagnostic Systems, Branchburg, N.J.) has been evaluated by our laboratory for female and male urine specimens and found to be highly sensitive and specific in multiple studies (13,14). Published studies comparing PCR to a revised gold standard of a positive culture or another positive test including molecular amplification with different primers demonstrated sensitivities ranging from 89% (one site) to 98.6% and specificities from 99.7 to 100% (15). The one clinical site reporting a sensitivity of 89% for endocervical specimens reported that a labile inhibitor influenced the sensitivity (16). Our most recent study submitted for publication, which includes PCR results for cervical swabs as compared to culture for 418 women indicated that the sensitivity was 86% and the specificity was 99% (17). Our most recent data from a national CDC-funded prevalence study, using this assay for 5,955 female cervical specimens from STD patients, indicated an increase in prevalence from 7.8% by culture in 1993 to 13.4% by PCR in 1994-1995 (Gaydos, unpublished data). In this same prevalence study, 2861 cervical specimens from 2 family planning clinics had a positivity rate of 8.0%

Recently, self-administered vaginal swabs (SAS), a novel specimen type, were evaluated by PCR for chlamydia infection. Even though chlamydial organisms infect the cervical columnar epithelial cells, the power of the amplified DNA technology (DNA from one organism is amplified one million-fold) is sufficient to detect those infected cells, which are exfoliated into the vaginal introitus. These self-administered vaginal swabs were tested by PCR and compared to cervical PCR as well as culture as the gold standard for 26 chlamydia positive women (18). Of the infected women, cervical culture detected 21 (81%), cervical PCR detected 22 (85%), vaginal introitus self administered detected 21 (81%), and vaginal introitus, nurse administered, detected 24 (92%). Similar recent studies reported SASs to be as sensitive as a speculum-obtained swab for PCR detection of chlamydia and gonorrhea cervical infections (19,20) and similar techniques have also been proven successful for trichomonas (21) and HPV detection (22,23).

Self-collected samples also appear to be suitable for HPV diagnosis. Morrison and co-workers (22) asked 25 women investigated at a colposcopy clinic to also provide a second cervical lavage sample collected at home with a MY-PAP kit for HPV diagnosis. Seventeen women (68%) complied with the request; 16 samples were satisfactory. 88% of the self-collected samples were positive by HPV-PCR. There was essentially complete concordance between results of home-collected samples and clinic samples. Fairley et al. (24) compared specimens collected from self-administered tampons with specimens collected at the clinic during a gynecological examination. In PCR tests of paired specimens from 48 women, 73% of cervical scrapes and 69% of tampons were HPV-positive. There was excellent correlation between HPV type-specific diagnoses in samples collected by the two methods. Moscicki (23) compared pairs of self-collected vaginal samples, obtained with dacron swabs, with samples collected during gynecological examinations. HPV was detected by non-amplification based assay. Seventy-four pairs were negative and 24 pairs were positive with identical results, in the two tests. In ten specimens which gave discordant results, the self-collected samples were as likely to be HPV-positive as the speculum-assisted samples.

Our laboratory stability studies for clinical and laboratory strains of *C. trachomatis* have been performed and indicate that the chlamydial DNA is stable for 14 days in the commercial Roche molecular Systems Amplicor, Chlamydia transporter. A positive patient sample was aliquoted in 11 tubes and two series of 11 transporter tubes were inoculated with 5 and 50 inclusion forming units (IFU) of *C. trachomatis* serovar E per tube. These tubes were set out at room temperature to simulate transport conditions. These transporter tubes contained sodium dodecyl sulfate, 0.4% (SDS) and TRIS buffer, 0.13%, so that potential nucleases which might degrade DNA would be expected to be inhibited. At days 0 to 14, specimens were removed from the room temperature rack and processed according to manufacture's directions: one millileter of treatment buffer (Tween 20), was added to the specimen to neutralize the SDS. The specimens were then refrigerated until the next PCR batch was performed (2-4 days). All of the room temperature stored tubes gave positive tests in the subsequent PCR tests, indicating that mailed or transported chlamydia-containing specimens would be stable for up to 14 days and could be detected as positive by PCR of the chlamydial DNA. In addition, laboratory studies from the Roche Molecular Systems using real patient specimens have indicated that specimens kept at room temperature for up to 33 days, still remained positive in the PCR test, as compared to culture. An

HPV-positive clinical specimen was employed to test the stability of HPV DNA in the transport medium in a way similar to that described above for chlamydia DNA. The HPV hybridization signal after PCR did not decrease in intensity through the observation period of 8 days.

Our laboratory stability studies for a fresh clinical isolate of gonorrhea indicate that the DNA from this isolate is stable in the Roche transporter for 11 days at room temperature. The transporter was inoculated with 10 organisms, as determined by plating serial 10-fold dilutions of a # 0.5 Barium Sulfate standard ( $10^8$  organisms/ml) and actual plate counts of the diluted specimens. The inoculated transporters were treated as above by incubating the tubes at room temperature and processing one per day. The stored and processed specimens were tested in the Roche multiplex Chlamydia/gonorrhea PCR test. The DNA remained positive in the PCR test for the maximum days tested (11) with no difference in optical density from day 0 (1.019) to day 11 (1.256). The test was positive for 100 organisms for this time period, but not for 1 organism.

Our laboratory has also conducted stability studies for a clinical specimen from a woman shown to be positive for trichomonas by wet mount preparation in a manner similar to that described above. Fifty microliters of the clinical swab specimen were added to the Roche transporter tubes, which were incubated at room temperature. Treated specimens were used for our in-house trichomonas PCR, and they were shown test positive, by giving the expected band on a silver stained polyacrylamide gel. Dilutional studies, using cytometer-counted trichomonas organisms, were shown to be able to detect both 50 and 5 trichomonas organisms per PCR. The trichomonas organism can also be detected in the multiplex system by adding trichomonas primers to the multiplex PCR mixture used for the chlamydia-gonorrhea assay. Further study may allow PCR multiplexing of all three organisms in the future, which could decrease the cost of the overall PCR testing protocol.

These SAS and specimen stability data indicate that this approach, namely a non-invasive, self-administered swab specimen for STD detection, has the potential to drastically improve STD screening in military women. During the first study year we have implemented and are testing this hypothesis. Furthermore, we hypothesize that for military women access to medical care through collecting and sending the specimen to a medical facility by mail or courier may be increased. Compliance to submit to STD screening may also be enhanced greatly, since a speculum examination of the vagina and cervix could be avoided, at least for the screening test. If we continue to demonstrate successful results, the use of SAS diagnostics for all common STDs could revolutionize the approach to efficient and cost effective STD testing for military women in an effort to reduce the morbidity associated with these diseases.

The objectives of this study are to

1. Establish prevalence of the above STDs among an estimated 1,275 consenting active-duty military women presenting to the Ft. Bragg Epidemiology and Disease Control (EDC) clinic
2. Determine sensitivity and specificity of clinician-administered intravaginal swab to detect the above STDs using PCR techniques compared to that obtained by standard diagnostic tests performed on specimens obtained during speculum-assisted gynecologic examination.
3. Determine the clinical profile of women with the above STDs .
4. Develop STD screening algorithm from the above clinical profile and risk factor analysis.
5. Determine the performance ease of patient-administered SAS among a subgroup of 275 women attending the EDC and consenting to study participation. Modify the collection kit and self-use instructions to improve utility.

## BODY

### Methods

#### Population and Specimens

The Epidemiology and Disease Control (EDC) Clinic at Fort Bragg is a fully equipped medical treatment facility. Average patient census for active duty females presenting with vaginal discharge and/or potential STD exposure has been approximately 100/month for the past 2.5 years. Using clinic-standard diagnostic tests (modified Thayer-Martin culture for *N. gonorrhoea*, Syva Microtrak EIA for *C. trachomatis*, and wet prep for *T. vaginalis*), 20% of these active duty females are confirmed to be infected with one or more of these STDs. True incidence of these infections is unquestionably higher due to the relative insensitivity of the tests available for routine patient care in this facility, but the estimated prevalence rates based on 1995 statistics are 5% of gonorrhea, 14% for chlamydia, and 3% for trichomoniasis. Tools for laboratory diagnosis of human papillomavirus infection are not available, but Pap smears will be performed in this clinic on study volunteers for the duration of this study. Since this clinic has a census of approximately 100 women per month a projected number of 1500 study subjects will be approached for enrollment over a 15 month period. Assuming a minimal enrollment acceptance rate of 85%, we will enroll 1275 women for Phase I.

During the first 15 months of this study, consecutive active duty military women, 18 to 59 years of age, attending the EDC clinic will be invited to participate in the study. This clinic evaluates walk-in or referred patients who present with genitourinary symptoms, are known contacts to an individual with a diagnosed STD, or who present for routine STD screening because of self-perceived risk. Any woman presenting to the EDC for these complaints will be approached for study enrollment. Women will be approached for enrollment by the study assistant during the time between registration and clinician evaluation so as not to interfere with regular clinic flow. All study information, as well as informed consent, will be relayed to the patient in a separate clinic room and in a confidential manner.

If the woman agrees to participate and signs the informed consent, she will be assigned a **unique, confidential study number which will be used on all questionnaires and study specimens**. The study clinician will administer a 10 minute standardized questionnaire. From our experience with previous studies, we have found that trained, gender-matched clinicians establish more open relationships with patients, and reduce patient reporting biases. We will train a study nurse and a study assistant to administer the questionnaire in a "standardized" fashion, without leading the patient or deviating from the printed word in any significant fashion. The brief questionnaire will be developed to collect data regarding demographics, menstrual and reproductive history, contraceptive practices, use of female hygiene products, access-to-care patterns, frequency and results of previous Papanicolaou (Pap) smears. Data regarding reason for visit, symptom type and duration, and antibiotic use during the previous two weeks will be recorded. Information will also be collected to determine risk factors related to STDs and HIV, such as sexual orientation, numbers and types (casual, regular, anonymous) of sex partners and sexual practices (e.g. oral-genital, genital-genital, rectal-genital) in the previous year, complete history of prior STDs or gynecologic infection, drug or alcohol use pattern relative to sexual activity, and drug and alcohol

use pattern among sexual partners. The questionnaire will be reviewed by investigators on an ongoing basis to assure the integrity of the data. These data will be analyzed to identify behavior profiles which put military women at risk for STD acquisition and thus to further develop and refine educational and instructional information regarding the use of the SAS.

After questionnaire completion, all participants will receive a standard pelvic exam. Prior to starting the pelvic examination, **one vaginal swab** will be collected from each participant prior to insertion of the speculum. The EDC clinician will initially administer the SAS in order to determine the best technique and timing to obtain optimal SAS sensitivity and specificity performance, and thus determine procedures for patient self-insertion. The swab will be sent to the Johns Hopkins STD Research Laboratory for PCR for *C. trachomatis*, *N. gonorrhoea*, *T. vaginalis*, and HPV. No additional discomfort is anticipated during specimen collection. Following this specimen collection, the speculum will be inserted and routine diagnostic tests for these and other infectious agents will be obtained according to SOP. Vaginal swabs will be obtained for trichomonas culture, pH testing, wet preparation for bacterial vaginosis (BV) and trichomonad detection, and 10% potassium hydroxide (KOH) preparation for yeast detection. Cervical swabs will be obtained for chlamydia enzyme immunosorbant assay testing (EIA), gonorrhea culture, chlamydia PCR, HPV PCR and cervical Gram's stain. A cervical Pap smear will be obtained for to detect changes that may be consistent with HPV infection (e.g. koilocytosis).

The sensitivities of vaginally-obtained swabs will be calculated using the results from the standard collection method obtained by use of speculum. For specimens that are positive by the vaginal swab and negative by standard culture, FDA-approved PCR assays will be performed on the speculum-obtained swabs which will be saved to help resolve any discrepancies. Treatment of chlamydia, gonorrhea, and trichomonas will be based on the gold standard FDA licensed tests, will follow the SOP algorithms, and will be in accordance to CDC STD treatment guidelines (25).

## Demographics

Since specimen collection began, 182 women have consented for enrollment into this study. Of these 182 women, 28% were Caucasian, 66% were African American, and the remaining 6% were classified as other race/ethnicity. The mean age was 25.3 years. Women reported to the EDC mainly for evaluation of symptoms (74%), and other reasons included screening visit (20%) and treatment as an STD contact (4%). Detection of the specific STDs of interest are reported below.

## Laboratory Procedures: Methods, Results, and Discussion

### *Neisseria gonorrhoea* and *Chlamydia trachomatis*:

#### Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* using a multiplex PCR assay.

##### Methods:

The detection of chlamydia and gonorrhea from vaginal swabs was performed with the multiplex PCR ("combo") kit by Roche Diagnostics Systems, Branchburg, NJ. This assay has been submitted for Federal Drug Administration (FDA) approval, clinical trials having been just completed. Our laboratory was one of the multicenter sites. The assay kits are being provided to Johns Hopkins University under a Material Transfer Agreement until FDA approval is obtained. Results of the vaginal swab assays are being compared to those from cervical swabs, which are obtained from standard operating procedures at Ft. Bragg, i.e. chlamydia, enzyme immunoassay and gonorrhea, culture. Discrepant results will be resolved at the end of the study by performing cervical swab PCR (FDA approved assay) and Ligase Chain Reaction (LCR) (Abbott Laboratories, Abbott Park, IL) for chlamydia and LCR for gonorrhea.

In addition to testing the vaginal swabs that are placed in the Roche transport tubes containing transport buffer ("wet swab"), we have added an additional test component. A "dry vaginal swab" is now also mailed to us in a empty tube. Upon arrival in the Johns Hopkins University laboratory within four days of collection, the swab is placed into the usual transport tube and processed according to usual directions. We have added this component because of exciting information published after submission of the grant, which indicated that cervical swabs can be submitted in a dry state for chlamydia (26).

##### Results:

To date, matching results from Ft. Bragg (standard of care) and Johns Hopkins (research vaginal specimens) for 182 specimens are available.

Organism	Site	Total	Positive	Prevalence
Chlamydia	JHU	182	25	13.7%
	Bragg	182	23	12.65
Gonorrhea	JHU	182	5	2.7%
	Bragg	182	3	1.6%

Discrepant specimens for chlamydia are JHU+/Bragg- 6 and Bragg+/JHU- 4.  
for gonorrhea are JHU+/Bragg- 2 and Bragg+/JHU- 0.

Results are available for a few more specimens at JHU, as well as “wet” versus “dry”:

Organism	Swab type	Total	Positive	Prevalence
Chlamydia	“Wet”	196	27	13.8%
	“Dry”	196	26	13.3%
Gonorrhea	“Wet”	196	5	2.6%
	“Dry”	196	6	3.1%

### Discussion

Thus far, performance of the SAS for both *C. trachomatis* and *N. gonorrhoeae* has been superior to SOP as expected. Procedurally, collection, labelling of specimens, mailing and processing have gone smoothly and according to proposed schedule.

Furthermore, the “dry swab” technique has performed as well for chlamydia as the “wet swab” and slightly better for gonorrhea. This is exciting preliminary data which will be very important if these results persist as we accrue larger numbers of patient specimens. The ability to mail a self-administered vaginal swab in a dry state in a empty container (such as a plastic bag) for the detection of several genital STDs obviously has great potential for the Army. Women in the field who perceive themselves to be at risk for STDs or who are having genital symptoms can collect and mail such dry specimens quickly, easily and mail them confidentially without “biohazard” identification. Furthermore, handling of dry specimens which when tested give high sensitivity and specificity may prove more cost effective than SOP and current commercially available quick diagnostic methods.

### ***Trichomonal vaginalis:***

#### Methods -

**Trichomonal Strains.** One ATCC strain (#30001) and thirteen strains of *T. vaginalis* isolated by culture from patients attending a city STD clinic were used to assess the sensitivity of the PCR primer sets. Specificity was tested by use of other related *Trichomonas* spp, amoebae, flagellates, and cervico-vaginal organisms (*Dientamoeba fragilis* ATCC #30948, *Chilomastix sulcatus* ATCC # 50562, *T. tenax* ATCC #30207, *T. gallinae* ATCC # 30002, *C. trachomatis*, and *N. gonorrhoeae*, *Giardia lamblia*, *Entamoeba histolytica*).

**Samples.** Vaginal swabs (N= 182) were collected from women attending the EDC clinic at Ft Bragg, who were also sampled for trichomonas culture and wet preparation. Culture was performed in Diamonds modified media broth. (Inpouch TV, Biomed Diagnostics, San Jose, CA). Vaginal secretions with one drop of normal saline were examined under a microscope (100 X) to identify motile organisms by wet preparation. Urine samples were processed by the Chelex method to extract the DNA.

**PCR Primers.** Two new sets of primers targeting a conserved region of the beta-tubulin gene of *T. vaginalis* was designed and tested.

BTUB2 : 5' GCA TGT TGT GCC GGA CAT AAC CAT 3'  
BTUB7 : 5' TAC ACT CAA GCT CAC AAC ACC AAC A 3'

BTUB41 : 5' TAA CAT CCA GGC TCG TAA CAC A 3'  
BTUB61 : 5' GGC TGT TTC GTA CAT TTC G 3'

These was compared to a set of primers previously described in the literature, slightly modified to avoid primer-dimer formation.

TVA5-1: 5' ATG TTC TAT CTT TTC ATT GT 3'  
TVA6 : 5' GAT CAC CAC CTT AGT TTA CA 3'

**PCR.** PCR reactions were performed with an automated thermocycler (Perking-Elmer Cetus, Norwalk, CT) using standard methodology. A touch down method for thermal cycling was used to improve specificity. Cycling times were 75 sec. at 94 °C followed by 60 cycles: denaturation temperature 94 °C for 45 sec., annealing temperature beginning at 60 °C and ending at 50 °C for 45 sec. and extension temperature of 72 °C for one min. The annealing temperature was lowered one degree every four cycles until reaching 50 °C, and then this annealing temperature was kept until the end of the cycling process.

**Detection of amplified targets:** The primers set BTUB 2 / 7 were designed to amplify a DNA segment of 76 base pairs (bp) and primer set BTUB 41/61 a product of 241 bp from the beta-tubulin gene. Primers TVA5-1 / 6 amplified a DNA segment of 102 bp from a non identified region in the *T. vaginalis* genome (clone A6p). Twenty µl of amplified products were electrophoresed at 120 volts in 12 % acrylamide bis-acrylamide gels in TBE buffer and stained with ethidium bromide (0.01 mM). The size of the product amplified was determined by comparison with commercial 50 bp weight marker XIII (Boehringer).

#### Results.-

Primer set BTUB 2 / 7 amplified a product of 76 bp in all fourteen *T. vaginalis* strains tested. Primer set BTUB 41/61 amplified a product of 241 bp in 13/14 strains of *T. vaginalis* tested; Strain M was not amplified. Primer set TVA5-1 / 6 amplified a product of 102 bp in 13/14 strains of *T. vaginalis* tested; strain L of *T. vaginalis* was not amplified. In addition primer set BTUB 2 / 7 and BTUB 41/61 amplified a 76 bp and 241 bp target from *T. tenax* and *T. gallinacea*. No amplified product was observed with DNA from other Trichomonas ssp with primer set TVA5-1/6. No target product was amplified from other vaginal pathogens or protozoa tested with either set of primers.

Sensitivity testing was assayed with strain M of *T. vaginalis* isolated in the STD Clinic. Two fold dilutions in culture media, beginning with 128 trichomonas organisms to one organism per PCR reaction were processed separately using Chelex to extract the DNA. All set of primers amplified one organism per PCR.

Twelve of the 182 vaginal samples (6.6%) were positive for *T. vaginalis* by either culture and/or wet preparation. Six of these positive samples were detected by PCR with set of primer TVA 5-1/6 (Appendix, Figure 1).

Because sensitivity was low, new sets of primers were investigated. Subsequently, the first 126 samples were compared using the three different set of primers. Seven of the 126 urine samples (5.5%) were positive for *T. vaginalis* by either culture and/or wet preparation (Appendix, Figure 2). Twenty-one specimens were PCR positive using primer set BTUB 2/7. Fourteen of these 21 samples were culture and wet preparation negative. Eight of these discrepant samples were confirmed as true positives by prime sets TVA 5-1/6 targeting a different region of *T. vaginalis* genome and/or BTUB 41/61 targeting a different region of the beta-tubuline gene (Appendix, Figure 3).

After resolving discrepant results, the sensitivity of the BTUB 2 / 7 primers was 94% and the specificity 95% (Appendix, Figure 4). The final prevalence for this population after discrepant analysis was 12.7 % (16/126).

### Discussion.

These preliminary results indicated that primer set BTUB 2 / 7 is sensitive set of primers for the detection of *T. vaginalis* in urine specimens. They were very sensitive, being able to amplify one trichomonas organism per PCR reaction. The primer set BTUB 2 / 7 amplified a 76 bp target in the beta-tubulin gene in all strains of *T. vaginalis* tested.

In the genome of *T. vaginalis* there are several copies of three genes encoding beta-tubulin proteins (beta-tubulin 1 ,2, and 3). Primer set BTUB 2 / 7 was designed to detect a well preserved region in all three genes to increase sensitivity. This region appeared to be preserved across species since *T. tenax* and *T. gallinacea* , which are not found in the vagina, where also detected by this set of primers. Other protozoa and vaginal organism where not detected.

The specificity of the primers depended on the annealing temperature. The use of Ampli-Taq Gold Polymerase avoided non-specific annealing of the primers because this enzyme is not active until heated to 94 °C, simulating a hot start. The addition of a touch down protocol increased the specificity of PCR by favoring the amplification of targeted copies of DNA polymerized during early cycles at higher annealing temperatures.

The Chelex method for DNA extraction appeared to work well for sample preparation. Since it is a resin, it may bind inhibitors and cellular debris present in the sample.

PCR (BTUB 2/7) detected all specimens that were positive by wet preparation and/or culture. After resolving discrepant, one specimen that was positive by primer sets TVA 5-1/6 and BTUB 41/61 was negative by primer set BTUB 2/7.

Promising preliminary results for the detection of *T. vaginalis* by PCR in vaginal specimens were shown in this study. Dry swabs have not yet been tested. Vaginal samples, which could be

obtained by the patient herself, are easier to obtain than endocervical specimens, thus providing a practical method for screening women for trichomonas. Primer set BTUB 2/7 for the detection of *T. vaginalis* in self-collected vaginal samples could be incorporated into a strategy for diagnosis of several STDs by PCR providing a cost effective way to screen populations at risk for sexually transmitted diseases. These data will be submitted in abstract form for the 1998 American College of Microbiologists Meeting in Atlanta.

### ***Human papilloma virus:***

#### Methods

The endocervical, "wet" SAS, and "dry" SAS specimens from each of seventy three subjects were amplified by PCR for the detection of papillomaviruses using the MY09/MY11 consensus primers in conjunction with the PC04/HG20  $\beta$ -globin control primers. Ethidium bromide staining of the resulting products indicated excellent amplification, with numerous HPV positive samples. However, it was noted that after transfer to nylon membranes, the products hybridized poorly with both HPV and  $\beta$ -globin control probes. It has been determined that the detergents in the neutralized transport medium interfere with the transfer to nylon, and we are addressing this problem.

#### Results

While the hybridizations were weak, we are still able to draw conclusions from the first tests. Thirty six of the subjects tested positive for HPV or  $\beta$ -globin in all specimens making them acceptable for analysis. Of these, 14 had all three of their specimens positive for HPV and 14 had all three negative. The other 8 had two of three specimens HPV positive, with the dry vaginal swab negative three times, and the cervical swab being negative 5 times. HPV type agreement between the three specimens is shown in table #1 for the 22 patients that were HPV positive. Many of the specimens were positive for the generic probe only (55%) and this is due to the poor transfer mentioned above. This data will change as we resolve that problem. Still we can see excellent agreement between the three specimens from each individual.

The three types of specimens from each individual amplified with approximately equal efficiency (table #2). Individual HPV types were identified nine times in both the STM and dry vaginal swabs, and twelve times in the cervical, probably not a significant difference.

#### Discussion

These preliminary data are promising in verification of this method using Roche Transport for HPV detection. We will be going to analyse this information as it relates to the results of routine PAP smear reports obtained by the EDC.

## **DISCUSSION AS TO STATEMENT OF WORK**

**Task 1: Proposed:** Months 1-3: Hire and train Ft. Bragg Study Nurse. Develop and duplicate study questionnaire

**Completed:** Nurse trained and study integrated into the regular clinic flow

**Task 2: Proposed:** Months 4-18: Enroll approximately 85 active duty military women per month attending the Epidemiology and Disease Control (EDC) Clinit at Ft. Bragg.

**Completed:** From March thru 9/5/97 a total of 196 women have volunteered for an average of 40/month. This number is below our target and due largely to the fact that the EDC lost a clinician, one study nurse left and another had to be trained. The position was only recently filled and training of the new personnel is in progress.

It is expected that with the personnel in place, 20 women can be enrolled on a weekly basis and we can continue to meet our expectation of 1,250 women.

**Task 3: Proposed:** Months 4-18: Determine sensitivity and specificity of the SAS to detect gonorrhea, chlamydia, trichomonas, and human papilloma virus infections using PCR techniques compared to that obtained by standard diagnostic tests and selected gold standard tests performed on specimens obtained during speculum-assisted gynecologic examination.

**Completed:** Sensitivity and specificity analyses are underway as reported.

## REFERENCES

1. Centers for Disease Control and Prevention. Recommendations for the prevention and management of *Chlamydia trachomatis* infections. MMWR 1993; 42:1-39.
2. Quinn, T.C., Zenilman, J. and Rompalo, A. Advances in diagnosis and treatment. In: *Sexually Transmitted Diseases*, edited by Schrier, R.W., Abboud, F.M., Baxter, I.D. and Fauci, A.S. St. Louis: Mosby, 1994, p. 149-196.
3. Boyd, R.S. and DeMaio, J. Use of Chlamydiazyme on urine sediment for diagnosis of *Chlamydia trachomatis* genital infections. Mil Med 1991; 156:420-421.
4. Malone, J.D., Hyams, K.C., Hawkins, R.E., Sharp, T.W. and Daniell, F.D. Risk factors for sexually-transmitted diseases among deployed U.S. military personnel. Sex Transm Dis 1993;294-298.
5. Catterson, M.L. Prevalence of asymptomatic chlamydial cervical infection in active duty army females. Mil Med 1993; 158:618-619.
6. Orndorff, G.R. Screening for *Chlamydia trachomatis* by the direct fluorescent antibody test in female Navy recruits. Mil Med 1991; 156:675-677.
7. Pfaff, J.A. and Pimentel, L. Chlamydial antigen testing on female patients presenting to the emergency department. Mil Med 1991; 156:362-364.
8. Centers for Disease Control and Prevention. *Sexually transmitted diseases surveillance*, Atlanta: Department of Health & Human Services, 1993. pp. 1-103.
9. Goodhart, M.E., Ogden, J., Zaidi, A.A. and et al. Factors affecting the performance of smear and culture tests for the detection of *Neisseria gonorrhoeae*. Sex Transm Dis 1982; 9:63-69.
10. Goh, B.T., Varia, K.B., Ayliffe, P.F. and et al. Diagnosis of gonorrhea by gram-stained smears and cultures in men and women: role of the urethral smear. Sex Transm Dis 1985; 12:135-138.
11. Schachter, J., Stamm, W.E., Chernesky, M.A., et al. Nonculture tests for genital tract chlamydial infection. What does the package insert mean, and will it mean the same thing tomorrow. Sex Trans Dis 1992; 19:243-244.
12. Stamm, W.E. and Holmes, K.K. *Chlamydia trachomatis infections of the adult*, New York: McGraw-Hill Co, 1990. pp. 181-193.
13. Jaschek, G., Gaydos, C., Welsh, L. and Quinn, T.C. Direct detection of *Chlamydia trachomatis* in urine specimens from symptomatic and asymptomatic men by using a rapid polymerase chain reaction assay. J Clin Microbiol 1993; 31:1209-1212.
14. Jones, K., Gaydos, C., Welsh, L., Lentz, A., Pare, B., Zenilman, J. and Quinn, T.C. 1994. Detection of *Chlamydia trachomatis* in male and female urine specimens and endocervical specimens using polymerase chain reaction. Ann Meet Amer Soc Microbiol. C443:569(Abstract)

15. Quinn, T.C. Recent advances in diagnosis of sexually transmitted diseases. Sex Trans Dis 1994; 21:S19-S27.
16. Bauwens, J.E., Clark, A.M. and Stamm, W.E. Diagnosis of *Chlamydia trachomatis* endocervical infections by a commercial polymerase chain reaction assay. J Clin Microbiol 1993; 31:3023-3027.
17. Quinn, T.C., Welsh, L., Lentz, A., et al. Diagnosis by Amplicor PCR for *Chlamydia trachomatis* infection in urine samples from women and men attending sexually transmitted disease clinics. J Clin Micro 1996; 34:1401-1406.
18. Wiseenfeld, H.C., Heine, R.P., DiBiasi, F.M., Repp, C.A., Rideout, A., Macio, I. and Sweet, R.L. 1995. Self collection of vaginal introitus specimens: A novel approach to *Chlamydia trachomatis* testing in women. Eleventh Mtg. of the International Society for STD Research. #040:(Abstract)
19. R.P.Heine, H.Wiesenfeld, F.Dibiasi, S.Witkin, A.Rideout, I.Macio and R.L.Sweet. 1995. The Vaginal Introitus: A Non-Invasive testing site for *Trichomonas vaginalis*. ICAAC. K87:303(Abstract)
20. H.C.Wisenfeld, R.P.Heine, A.Rideout, I.Macio, F.Dibiasi, A.Reep and R.L.Sweet. 1995. The Distal Vagina is a novel site for gonorrhea (GC) testing. ICAAC. K86:303(Abstract)
21. Wawer, M.J., McNairn, D., Wabwire-Mangen, F., Paxton, L., Gray, R.H. and Kiwanuka, N. Self-administered vaginal swabs for population-based assessment of *Trichomonas vaginalis* prevalence. Lancet 1995; 345:131-132.
22. Morrison, E.A.B., Goldber, G.L., Hagan, R.J., Kadish, A.S. and Burk, R.D. Self-administered home cervicovaginal lavage: A novel tool for the clinical-epidemiologic investigation of genital human papillomavirus infections. Am J Obstet Gynecol 1992; 167:104-107.
23. Noscicki, A.B. Comparison between methods for human papillomavirus DNA testing: A model for self-testing in young women. J Infect Dis 1993; 167:723-725.
24. Fairley, C.K., Chen, S., Tabrizi, S.N., Quinn, M.A., McNeil, J.J. and Garland, S.M. Tampons: A novel patient-administered method for the assessment of genital human papillomavirus infection. J Infect Dis 1992; 165:1103-1106.
25. Centers for Disease Control and Prevention. 1993 *Sexually transmitted diseases treatment guidelines*, Atlanta: Epidemiology Program Office, 1993. Ed.42th pp. 1-102.
26. Kellogg, J.A., Seiple, J.W., Klinedinst, J.L., Stroll, E.S. and Cavanaugh, S.H. Improved pcr detection of *Chlamydia trachomatis* by using an altered method of specimen transport and high-quality endocervical specimens. J Clin Microbiol 1995; 33:2765-2767.

## **APPENDIX**

**Figure 1** PCR results of primer set TVA 51/6 in 182 urine samples compared to wet preparation and / or culture of *Trichomonas vaginalis*

		Wet preparation or culture			
		+	-		
Primer	+	6	6	12	<div> Sens. % : 50  Spec. % : 96  PPV % : 50  NPV % : 96 </div>
TVA 51/6	-	6	164	170	
		12	170	182	

**Figure 2** Comparison of PCR results with primer sets TVA 51/6, BTUB 2/7, and BTUB 41/61 for the diagnosis of *Trichomonas vaginalis*

		Wet preparation or culture			
		+	-		
Primer	+	4	6	10	<div> Sens. % : 57  Spec. % : 95  PPV % : 40  NPV % : 97 </div>
TVA 51/6	-	3	113	116	
		7	119	126	

		Wet preparation or culture			
		+	-		
Primer	+	7	14	21	<div> Sens. % : 100  Spec. % : 88  PPV % : 33  NPV % : 100 </div>
BTUB 2/7	-	0	105	105	
		7	119	126	

		Wet preparation or culture			
		+	-		
Primer	+	3	6	9	<div> Sens. % : 43  Spec. % : 95  PPV % : 33  NPV % : 97 </div>
BTUB 41/61	-	4	113	117	
		7	119	126	

**Figure 3** Comparison of results by culture or wet preparation and PCR using primer sets: TVA 51/6, BTUB 2/7, and BTUB 41/61.

Number of samples	Wet preparation or culture	PCR primers		
		TVA 51/6	BTUB 2/7	BTUB 41/61
1	+	+	+	+
3	+	+	+	-
2	+	-	+	+
1	+	-	+	-
1	-	+	+	+
3	-	+	+	-
4	-	-	+	+
1	-	+	-	+
1	-	+	-	-
6	-	-	+	-
103	-	-	-	-
Total positives	7	10	21	9

**Figure 4** Results of PCR for the detection of *Trichomonas vaginalis* by PCR after resolving\* discrepant results with culture and wet preparation.

After discrepant results resolved						
		+	-			
Primer TVA 51/6	+	9	1	10	Sens. % :	56
	-	7	109	116	Spec. % :	99
		16	110	126	PPV % :	90
					NPV % :	94
After discrepant results resolved						
		+	-			
Primer BTUB 2/7	+	15	6	21	Sens. % :	94
	-	1	104	105	Spec. % :	95
		16	110	126	PPV % :	71
					NPV % :	99
After discrepant results resolved						
		+	-			
Primer BTUB 41/61	+	9	0	9	Sens. % :	56
	-	7	110	117	Spec. % :	100
		16	110	126	PPV % :	100
					NPV % :	94

\* discrepant results were resolved as true positives when samples were detected positive by two or more sets of primers.

Table #1. HPV POSTITIVE DATA FOR PATIENTS  
IN WHICH ALL THREE SPECIMENS WERE SATISFACTORY

Patient No.	HPV Type Vag./STM	HPV Type Vag./Dry	HPV Type Cervical/STM
2	16,51	51	16,51
3	GEN	GEN	PAP291
5	CP8304	CP8304	CP8304
6	GEN	GEN	NEGATIVE
10	GEN	GEN	NEGATIVE
11	GEN	GEN	GEN
13	GEN	GEN	6
22	18	18	6,18
23	GEN	NEGATIVE	GEN
25	GEN	GEN	GEN
31	CP4173	GEN	NEGATIVE
36	58	58	58
40	GEN	NEGATIVE	58
43	GEN	GEN	GEN
46	GEN	GEN	56,58
49	GEN	6	GEN
55	GEN	GEN	6
60	53,66	53	53,66
66	GEN	NEGATIVE	52
68	16,31,68	16,31,68	16,31,68
70	31	31	NEGATIVE
72	31	31	NEGATIVE

Table #2. POSITIVE HYBRIDIZATIONS FOR THE  
THREE TYPES OF SPECIMENS COLLECTED

	Vag./STM	Vag./Dry	Cervical/STM
$\beta$ -globin probe	44	43	59
Generic probe	30	22	22
$\beta$ -globin + Generic	21	16	21